Attorney Docket No. REGEN1260-3

In re Application of Tsien et al.

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Amendments to the Claims

Please amend claims 59, 60 and 61 as indicated in the listing of claims.

Please cancel claims 57, 58 and 76 without prejudice or disclaimer.

The listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claims 57-58. (Canceled)

- 59. (Currently Amended) The construct of claim <u>79, 80 or 81 57 or 58</u>, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.
- 60. (Currently Amended) The construct of claim 79, 80 or 81 57 or 58, wherein the donor moiety acceptor moiety and the linker moiety are fused in a single amino acid sequence.
- 61. (Currently Amended) The construct of claim 79, 80 or 81 57 or 58, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV-1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, b-Secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claims 62-75. (Withdrawn)

- 76. (Canceled)
- 77. (Withdrawn)
- 78. (Withdrawn)

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- 79. (New) A tandem fluorescent protein construct comprising:
 - i) a donor fluorescent protein moiety comprising an amino acid sequence substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by amino acid substitutions selected from the group consisting of:
 - a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys;
 - b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr;
 - c) Tyr66His and Tyr145Phe;
 - d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;
 - e) Ser72Ala, Tyr145Phe and Thr203Ile; and
 - f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr;
 - ii) an acceptor fluorescent protein moiety comprising an amino acid sequence substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by amino acid substitutions selected from the group consisting of:
 - a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr; and
 - b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr; and
 - iii) a linker moiety that couples the donor moiety of i) and the acceptor moiety of ii), wherein the linker moiety comprises a protease recognition site.
- 80. (New) A tandem fluorescent protein construct comprising:
 - i) a donor fluorescent protein moiety comprising an amino acid sequence substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by amino acid substitutions selected from the group consisting of:
 - a) Tyr66His and Tyr145Phe; and
 - b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Ans212Lys;

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- ii) an acceptor fluorescent protein moiety comprising an amino acid sequence substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by amino acid substitutions selected from the group consisting of:
 - a) Ser65Cys; and
 - b) Ser65Thr; and
- iii) a linker moiety that couples the donor moiety of i) and the acceptor moiety of ii), wherein the linker moiety comprises a protease recognition site.
- 81. (New) A tandem fluorescent protein construct comprising:
 - A) a donor fluorescent protein moiety comprising:
 - i) an amino acid sequence substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by amino acid substitutions selected from the group consisting of:
 - a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys;
 - b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr;
 - c) Tyr66His and Tyr145Phe;
 - d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;
 - e) Ser72Ala, Tyr145Phe and Thr203Ile; and
 - f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr; or
 - ii) an amino acid sequence substantially identical to SEQ ID NO:2 and comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223, wherein the mutation attenuates the intermolecular interactions between the donor and acceptor moieties;
 - B) an acceptor fluorescent protein moiety comprising:
 - i) an amino acid sequence substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by amino acid substitutions selected from the group consisting of:

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- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr; and
- b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr; or
- an amino acid sequence substantially identical to SEQ ID NO:2 and comprising a mutation that reduces the hydrophobicity at positions A206,
 L221 or F223, wherein the mutation attenuates the intermolecular interactions between the donor and acceptor moieties; and
- C) a linker moiety that couples the donor moiety of A) and the acceptor moiety of B), wherein the linker moiety comprises a protease recognition site.